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ESTIMATION OF CLEAN-FLEECE WEIGHT FROM GREASE-FLEECE WEIGHT AND STAPLE LENGTH [1]

By CLAIR E. TERRILL, *animal husbandman*, ELROY M. POHLE, *associate animal fiber technologist*, L. OTIS EMIK, *junior animal husbandman*, and LANOY N. HAZEL, *assistant animal husbandman, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

Clean-fleece weight is the most important measure of fleece value in breeding for improvement of sheep. This is particularly true within breeds when variations in grade and quality of wool are not great. Although most wool in the United States is sold on a grease-wool basis, the price is usually determined by the buyer's estimation of the amount of clean wool present. If this estimation is accurate, weight of clean wool is one of the most important factors affecting the income from wool.

The amount of clean wool per fleece can be determined exactly by scouring the entire fleece or approximately by scouring a representative sample of each fleece. However, even the latter practice requires much routine work when large numbers are involved, and adequate equipment and personnel are seldom available to carry on this routine continuously. This condition has prompted the search for methods that would make it possible to estimate clean-fleece weight from other fleece characters with a high degree of accuracy.

Correlations between various fleece characters of range Rambouillet sheep have been studied by Spencer and coworkers (*7, p. 45*).[2] They concluded:

> The scoured-fleece weights became greater as the weights became greater in the unscoured fleeces, and in the moisture, grease, and dirt per fleece. The length of staple was generally longer, the fineness a trifle less, and the character and density of the fleeces slightly more excellent as the yields of clean wool per fleece increased.

These writers showed that the weight of unscoured (grease) wool was the best factor for indicating the weight of scoured (clean) wool per fleece. Pohle and Keller (*4*), working with range Rambouillet, Targhee, Columbia, and Corriedale yearling ewes, found that with each centimeter (three-eighths inch) of increase in staple length there was an increase of about one-fourth to one-half pound, or almost 1 to 2.5 percent, of clean wool. Length of staple had a greater influence on clean-fleece weight than did body weight.

Lambert and coworkers (*2*) showed that fleece length in weanlings had some predictive value for estimating clean-fleece weight in yearlings in the Corriedale, Columbia, and Rambouillet breeds. Pohle (*3*) found in the same breeds that length of staple and percentage of clean wool in weanlings had a high relationship with these same characters in yearlings.

[1] Received for publication July 8, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 10.

Previous work [3] with 186 Rambouillet ewes showed that fleece length and body weight at weanling or yearling ages were reliable in estimating clean-fleece weight in yearlings. The multiple correlation coefficients were 0.71 and 0.68 for weanling and yearling characters, respectively. Fleece length was more important than body weight in estimating clean-fleece weight, particularly in yearlings. The correlation coefficients of grease-fleece weight, fleece length, and body weight with clean-fleece weight were 0.69, 0.63, and 0.37, respectively, in yearlings. The multiple correlation coefficient between clean-fleece weight and the three variables was 0.786, as compared with 0.783 when body weight was omitted. Therefore, in yearlings, body weight did not add to the ability to estimate clean-fleece weight, probably owing to a fairly high relationship between body weight and grease-fleece weight. Other measures, such as fleece-character score, density score, density index, and fleece fineness at the side and thigh, had such low relationships with clean-fleece weight that they could have little value in its estimation.

The objects of this study, which involved several years' data from fairly large numbers of sheep of four breeds, were: (1) To develop multiple regression equations for the estimation of clean-fleece weight from grease-fleece weight and staple length; (2) to test the reliability of these equations; (3) to present a nomograph for the rapid estimation of clean-fleece weight; and (4) to develop formulas for annual corrections for variations in grease-fleece weight, staple length, and clean-wool yield. The work was conducted at the Western Sheep Breeding Laboratory and United States Sheep Experiment Station, Dubois, Idaho

MATERIAL AND METHODS

Data on grease-fleece weight, staple length, and clean-fleece weight from 1,037 Rambouillet, 253 Targhee, 214 Corriedale, and 211 Columbia yearling ewes were taken during the 3-year period from 1939 to 1941. These were fairly normal years with respect to clean-wool yield or shrinkage. In 1941 there were two groups of Rambouillet yearlings. Group A consisted of inbreds and group B of outbreds. The former animals were born a month earlier and were managed somewhat differently than the latter. The data for the two groups, therefore, are presented separately.

Just prior to shearing, staple length was measured near the middle of the side of each ewe to the nearest 0.2 cm., the average of three measurements by different judges being used as the actual length. Grease-fleece weight was taken on the shearing floor to the nearest 0.05 pound. Clean-wool yields, in terms of percentage, were determined by scouring a small sample of wool from each ewe. The small sample, weighing 25 to 35 gm., was obtained with the use of an electric clipper from an area about 11 cm. long and 5 cm. wide near the middle of the side. The samples were immediately placed in individual moisture-proof containers, being scoured later as described by Hardy (*1*) to obtain the bone-dry clean-wool yields. The term "bone-dry" refers to wool from which practically all the moisture had been eliminated by drying in a conditioning oven at 212° F. for a period of 6 hours. The clean-wool yield of the whole fleece was calculated from that of the small sample by the method described by Schott and coworkers (*5*).

[3] TERRILL, CLAIR E. Unpublished data.

The clean-fleece weight was then obtained by multiplying the grease-fleece weight by the calculated clean-wool yield of the whole fleece. Grease-fleece weights, staple lengths, and clean-fleece weights were taken at about 400 days' growth and were adjusted to 365 days' growth

$$\text{(adjusted measurement} = \frac{\text{actual measurement}}{\text{actual days growth}} 365)$$

to minimize age differences.

Clean-fleece weights were also obtained from scoured whole fleeces of 85 Rambouillet, 46 Targhee, 55 Corriedale, and 55 Columbia yearling ewes. These fleeces were sent to the Agricultural Marketing Administration of the United States Department of Agriculture where they were scoured by the method described by Buck.[4]

DATA AND DISCUSSION

The general relationships among grease-fleece weight, staple length, and clean-fleece weight by breeds and years are shown by the averages, correlation coefficients, and standard partial regression coefficients in table 1. Correlations were highest between grease-fleece weight and clean-fleece weight and were lowest between grease-fleece weight and staple length. In every case grease-fleece weight was more important than staple length for the estimation of clean-fleece weight. However, staple length became progressively more important in the breeds with shorter staple and finer grades of wool.

TABLE 1.—*Average grease-fleece weight, staple length, and clean-fleece weight with correlation and standard partial regression coefficients for 4 breeds of sheep during a 3-year period*

Breed	Year data were taken	Sheep	Averages [1]			Correlation coefficients			Standard partial regression coefficients	
			Grease-fleece weight (X_1)	Staple length (X_2)	Clean-fleece weight (Y) [2]	r_{Y1}	r_{Y2}	r_{12}	$\beta_{Y1.2}$	$\beta_{Y2.1}$
		Number	*Pounds*	*Centimeters*	*Pounds*					
Rambouillet [3]	1939	195	8.29	5.65	2.77	0.78	0.57	0.40	0.65	0.31
	1940	272	9.11	5.68	3.09	.74	.67	.52	.53	.39
	1941A	308	9.33	5.95	3.42	.74	.63	.34	.59	.44
	1941B	262	8.96	5.67	3.26	.69	.62	.37	.54	.42
Average		1,037	8.98	5.75	3.17	.73	.63	.41	.57	.40
Targhee	1939	74	9.64	6.80	3.40	.73	.63	.41	.57	.39
	1940	91	10.33	7.34	4.07	.81	.62	.36	.67	.37
	1941	88	9.59	7.61	3.77	.79	.64	.45	.62	.36
Average		253	9.87	7.29	3.77	.78	.63	.41	.63	.37
Corriedale	1939	64	9.50	8.47	3.66	.72	.72	.46	.50	.49
	1940	72	9.38	8.36	3.83	.81	.61	.59	.69	.20
	1941	78	9.50	8.97	4.05	.83	.59	.35	.71	.35
Average		214	9.46	8.61	3.86	.79	.63	.46	.64	.34
Columbia	1939	61	9.86	7.89	3.78	.78	.59	.38	.65	.35
	1940	88	10.81	8.24	4.55	.77	.52	.30	.68	.32
	1941	62	10.61	8.59	4.27	.84	.59	.46	.72	.26
Average		211	10.47	8.24	4.24	.79	.56	.38	.68	.30

[1] All grease-fleece weights, staple lengths, and clean-fleece weights are for 365 days' growth.
[2] Clean-fleece weights are based on bone-dry yields.
[3] The two 1941 Rambouillet groups were kept separate because group A was inbred, born a month earlier, and managed somewhat differently than group B.

[4] BUCK, W. M. PROGRESS IN WOOL SHRINKAGE RESEARCH DURING YEAR 1939. U. S. Dept. Agr., Agr. Market. Serv. 32 pp. [Processed.] 1940.

Multiple regression and correlation coefficients for estimating clean-fleece weight from grease-fleece weight and staple length are given in table 2. The similarity of the multiple correlation coefficients, their range being from 0.80 to 0.89, indicates that the ability to estimate clean-fleece weight from the two factors did not vary much from year to year or from breed to breed.

TABLE 2.—*Multiple regression and correlation coefficients for estimating clean-fleece weight (Y) from grease-fleece weight (X_1) and staple length (X_2)*

Breed	Year data were taken	Regression coefficients			Correlation coefficient
		a	$b_{Y1\cdot2}$	$b_{Y2\cdot1}$	R
Rambouillet [1]	1939	−0.88	0.2644	0.2585	0.83
	1940	−.83	.2240	.3315	.81
	1941A	−1.14	.2519	.3712	.84
	1941B	−1.76	.2865	.4315	.80
Average within years		−1.10	.2513	.3500	.81
Targhee	1939	−1.78	.2792	.3665	.82
	1940	−1.74	.3391	.3124	.88
	1941	−1.42	.2934	.3118	.85
Average within years		−1.62	.3075	.3226	.85
Corriedale	1939	−1.98	.2573	.3770	.84
	1940	−.09	.2968	.1360	.83
	1941	−1.03	.3198	.2273	.89
Average within years		−.92	.2923	.2344	.85
Columbia	1939	−1.12	.2871	.2623	.84
	1940	−1.57	.3687	.2585	.83
	1941	−1.32	.3571	.2100	.87
Average within years		−1.35	.3420	.2442	.84

[1] The two 1941 Rambouillet groups were kept separate because group A was inbred, born a month earlier and managed somewhat differently than group B.

The significance of differences between the means of the characters studied was tested by analysis of variance, as shown in table 3. Breed differences were highly significant in each case, whereas yearly differences were not significant. The highly significant breeds-×-years interaction indicates that the breeds react differently to yearly environmental changes. The means for the two 1941 Rambouillet groups differed significantly in all three characters.

TABLE 3.—*Analysis of variance for grease-fleece weight, staple length, and clean-fleece weight*

Source of variance	Degrees of freedom	Grease-fleece weight		Staple length		Clean-fleece weight	
		Mean square	F [1]	Mean square	F [1]	Mean square	F [1]
Total	1,714	2.38		2.00		0.64	
Breeds [2]	3	158.91	11.49**	766.17	97.85**	91.96	10.69**
Years [2]	2	48.86	3.53	8.95	1.14	21.51	2.50
Breeds × years	6	13.83	6.92**	7.83	12.63**	8.60	20.00**
Rambouillet groups	1	17.57	8.79**	11.18	18.03**	3.59	8.35**
Error	1,702	2.00		.62		.43	

[1] ** $P<0.01$, highly significant.
[2] Mean squares tested with the breeds-×-years interaction.

Tests for significant differences of the a values are presented in table 4. These consist in comparing clean-fleece weights adjusted for average breed, yearly, or interaction variations in grease-fleece weight and staple length. The a values were significantly different for breeds, years, and breeds-×-years interaction. Consequently, the a values in the multiple regression equation should be determined for each breed each year. The procedure for determining the a values is given later in this article in the application of results. The difference in average grease-fleece weight and staple length between the two 1941 Rambouillet groups accounted for practically all the difference in clean-fleece weight; hence, this test is not given in table 4.

TABLE 4.—*Tests for significant differences of a values*

Source of variance	Degrees of freedom	Sum of squares for clean-fleece weight (Sy^2)	R^2	Errors of estimate			
				Degrees of freedom	$Sy^2-R^2Sy^2$	Mean square	F [1]
Error	1,702	728.95	0.68264	1,700	231.34	0.1361	
Error+breeds	1,705	1,004.82	.76242	1,703	238.73		
Breeds				3	7.39	2.46	18.07**
Error+years	1,704	771.97	.67516	1,702	250.77		
Years				2	19.43	9.72	71.42**
Error+(breeds×years)	1,708	780.57	.69534	1,706	237.81		
Breeds×years				6	6.47	1.08	7.94**

[1] **$P<0.01$, highly significant.

Differences in the multiple regression coefficients were tested by an extension of the method given by Snedecor (*6*), as shown in table 5. Since the individual regressions for the two Rambouillet groups in 1941 were not significantly different, the average regressions ($b_{Y1\cdot2}$ and $b_{Y2\cdot1}$) were used for that year. The reduction in Sy^2 due to the average regression on grease-fleece weight or on staple length is shown in table 5. The additional reduction due to the four breed regressions was highly significant for both grease-weight and staple length, indicating that different regression coefficients should be used for each breed. Differences in yearly regressions were nearly significant, and those for the individual regressions for each breed each year were significant or highly significant. Breed differences in the regression coefficients were greater than yearly differences, but the breed regressions did not always occur in the same order. To estimate clean-fleece weight in succeeding years, it will be necessary to use the same regression coefficients each year. The above data indicate that these were not strictly homogeneous for the 3 years studied. However, the loss of information due to yearly variation in the actual regression coefficients is not likely to be large. In these data, errors of estimate of clean-fleece weight with the use of the average regression coefficients for each breed each year were 98.7 percent as large as the error of estimate with the use of the average regressions for each breed. Thus, the use of the four breed regression coefficients from year to year provides about 98.7 percent as much information as the calculation of new regression coefficients for each year. Likewise, the use of the average regression coefficients for all breeds and years provides about 97 percent as much information as the calculation of new regression coefficients for each breed each year. It seems worth while to use different

regression coefficients for each breed, as no additional work is required to estimate clean-fleece weights.

TABLE 5.—*Test of regression coefficients by years and breeds*

Source of variance	Degrees of freedom	X_1 with X_2 fixed			X_2 with X_1 fixed		
		Sum of squares	Mean square	F[1]	Sum of squares	Mean square	F[1]
Error	1,702	728.95			728.95		
Reduction due to other variable	12	279.94			420.94		
Deviation from other variable	1,690	449.01			308.01		
Reduction due to average regression	1	222.52			80.92		
Additional reduction due to yearly regressions	2	.71	0.355	2.69	.79	0.395	2.99
Additional reduction due to breed regressions	3	1.67	.557	4.22**	1.54	.513	3.89**
Additional reduction due to individual regressions	6	2.14	.357	2.70*	2.79	.465	3.52**
Errors of estimate	1,678	221.97	.132		221.97	.132	

[1] ** $P<0.01$, highly significant; * $P<0.05$, significant.

A practical test was made of the average within-year regression equations for each breed for estimating clean-fleece weight. Both small samples and whole fleeces of 241 yearling ewes were scoured, so that clean-fleece weight could be calculated in three ways for each breed: (1) By scouring the whole fleece (W); (2) by scouring a small sample (S); (3) by estimating from the within-year regression equations with grease-fleece weight and staple length as independent variables and S as the dependent variable (E).

The correlation coefficients between these variables are shown by breeds in table 6. Each of the multiple regression coefficients was calculated so the correlation between the dependent variable (S) and the estimated value (E) is a maximum. In all four breeds, values for r_{WE} were larger than those for r_{SE}, although the latter are similar to the multiple correlations given in table 2. This would be expected if the small samples were subject to unbiased errors, which would make S higher than the corresponding W in some cases and lower in others. The predictive value of grease-fleece weight and staple length for estimating clean-fleece weight, therefore, is higher than indicated by the multiple correlation coefficients involving these two factors and clean-fleece weight as determined from small samples.

TABLE 6.—*Correlation coefficients by breeds between clean-fleece weight, calculated by scouring the whole fleece (W), scouring a small sample (S), and estimating from grease-fleece weight and staple length (E)*

Breed	Fleeces	r_{WE}	r_{WS}	r_{SE}
	Number			
Rambouillet	85	0.86	0.89	0.80
Targhee	46	.89	.84	.82
Corriedale	55	.87	.91	.80
Columbia	55	.86	.94	.82
Average		.87	.90	.81

In table 6 the average for r_{WE} was only slightly less than for r_{WS}, indicating that clean-fleece weight can be estimated almost as accurately from grease-fleece weight and staple length as from scouring a small sample.

APPLICATION OF RESULTS

The average within-year regression equations, obtained by the use of the average regression coefficients for each breed given in table 2, were used in constructing the nomograph presented in figure 1. Thus

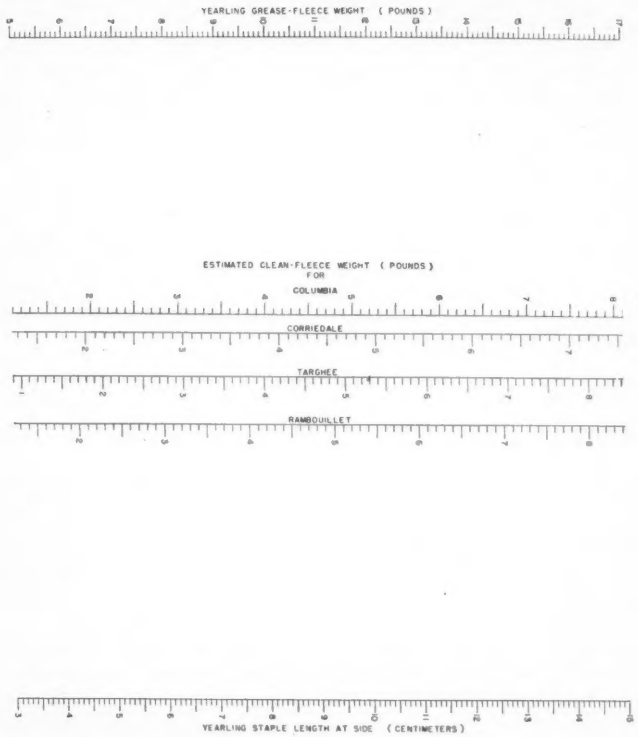

FIGURE 1.—A nomograph for the estimation of bone-dry clean-fleece weight from grease-fleece weight and staple length in four breeds of sheep. (Conversion may be made to an approximate commercial moisture basis by dividing the bone-dry weights by 0.88.)

by laying a ruler across the nomograph from a given grease-fleece weight on the left-hand scale to a given staple length on the right-hand scale, the estimated clean-fleece weight may be read directly from the middle scale for the respective breeds.

These clean-fleece weights will be adequate for use in a breeding program in which it is sufficient to rank individuals on clean-fleece weight each year. There will be yearly variations, however, in grease, dirt, moisture, and other foreign material that cannot be measured by a regression equation developed in previous years. Presumably variations of this kind were responsible for the significantly different a values as shown in table 4. In order to estimate clean-fleece weights that will be comparable from year to year and that will approach the

actual clean-fleece weights, it will be necessary to obtain the average percentage clean yields for each year. If small samples are scoured, percentage clean yield of whole fleeces (X) can be predicted from percentage clean yield of small samples (Y) by the simple regression equation $\frac{Y-a_{y\cdot x}}{b_{y\cdot x}}=X$, as shown by Schott and coworkers (*5*). With the same breeds used in this study, they found that $b_{y\cdot x}=1.08$ could be used for each of the four breeds. The values of $a_{y\cdot x}$ were -2.86, -1.62, -0.84, and 0.73 percent for Rambouillets, Targhees, Corriedales, and Columbias, respectively, for small side samples. Then the average clean weight of a group of whole fleeces can be obtained by multiplying the estimated percentage clean yield of the whole fleeces ($\overline{X}$) by the average grease-fleece weight ($\overline{G}$).

Annual variations in average clean-fleece weight, grease-fleece weight, and staple length can be expressed as deviations from the a values used in the multiple regression equations so that annual corrections for the a values may be applied directly to the estimated clean-fleece weights obtained with the nomograph. Only one correction will need to be calculated for each breed each year. These corrections for the a values are as follows:

Rambouillet $\left(\frac{\overline{Y}+0.0286}{1.08}\right)\overline{G}-0.2513\overline{G}-0.3500\overline{L}+1.10$

Targhee $\left(\frac{\overline{Y}+0.0062}{1.08}\right)\overline{G}-0.3075\overline{G}-0.3226\overline{L}+1.62$

Corriedale $\left(\frac{\overline{Y}+0.0084}{1.08}\right)\overline{G}-0.2923\overline{G}-0.2344\overline{L}+0.92$

Columbia $\left(\frac{\overline{Y}-0.0073}{1.08}\right)\overline{G}-0.3420\overline{G}-0.2442\overline{L}+1.35$

where $\overline{Y}$=average clean-wool yield from small side sample of a representative group of ewes, $\overline{G}$=average grease-fleece weight for current year, and $\overline{L}$=average staple length for current year.

For example, the correction for the a value for Corriedale yearling ewes in 1942 can be determined from the average clean-wool yield of 53.05 percent determined from small samples, the average grease-fleece weight of 7.9 pounds, and the average staple length of 10.1 cm. Thus the correction for the a value$=\left(\frac{0.5305+0.0084}{1.08}\right)(7.9)-0.2923(7.9)-0.2344(10.1)+0.92=0.2$. Therefore, 0.2 pound should be added to the clean-fleece weight estimated from the nomograph for each Corriedale yearling ewe in 1942. A Corriedale yearling ewe in 1942 with a grease-fleece weight of 9.8 pounds and a staple length of 10.4 cm. would have an estimated clean-fleece weight of 4.4 pounds as determined from the nomograph. The corrected clean-fleece weight would be 4.6 pounds. All estimated clean-fleece weights would be for 365 days' growth since grease-fleece weight and staple length should first be adjusted to this basis. The clean-fleece weights obtained may be converted from bone-dry weights to a commercial moisture basis by dividing by 0.88.

The number of ewes from each breed that would need to be sampled for clean-fleece yield each year can be estimated from data presented by Schott and coworkers (*5*). If entire fleeces were scoured, a rea-

sonable degree of accuracy could be obtained by scouring fleeces from 19 ewes selected at random for each breed. The standard error for the mean whole-fleece yield would then be

$$0.0075\left\{=\sqrt{\frac{\sigma x^2}{n}}=\sqrt{\frac{10.709}{19}}=0.75 \text{ percent}\right\}.$$

The same degree of accuracy could be obtained by scouring a representative small sample from each of 30 ewes

$$\left\{\sqrt{\frac{\sigma y^2}{(b_{y.x})^2(n)}}=\sqrt{\frac{19.449}{(1.1664)(30)}}=0.75 \text{ percent}\right\}.$$

It should be remembered that the regression equations presented in this paper apply only where conditions are similar to those near Dubois, Idaho. Marked differences in climatic conditions, plane of nutrition, or other environmental factors may require different a and b values for the regression equations. Although the method outlined is general, the specific application of these equations can be tested only by developing similar equations for flocks in widely separated regions. Wool laboratories maintained in most of the important sheep-producing States can perform valuable service in this respect, thereby placing the selection of improved breeding stock and the marketing of grease wool on a more reliable basis than is possible where individual clean-fleece weights and average percentage clean yield are not known.

SUMMARY

Multiple regression equations of clean-fleece weight, as determined from small samples, on grease-fleece weight and length of staple (taken from middle of side) were calculated on 1,037 Rambouillet, 253 Targhee, 214 Corriedale, and 211 Columbia yearling ewes during the 3-year period from 1939 to 1941. Multiple correlation coefficients for these four breeds were 0.81, 0.85, 0.85, and 0.84, respectively.

Grease-fleece weight was more important than staple length for the estimation of clean-fleece weight. However, staple length became progressively more important in the breeds with shorter staple and finer grades of wool.

Analysis of variance was used to test for differences between means, a values, and the multiple regression coefficients. Breed differences were highly significant, but yearly differences were not significant for the means for each of the three characters. The a values were significantly different for breeds, years, and breeds × years, indicating that the a values should be determined for each breed each year. Significant differences were found in the regression coefficients for breeds but not for years.

The multiple regression equations were tested on data from 241 of the yearling ewes for which whole fleeces had been scoured. The within-year regression equations for each breed appeared to be somewhat more accurate than indicated by the multiple correlation coefficients for the larger group of data for which clean-fleece weights were calculated by scouring small side samples. The accuracy of grease-fleece weight and staple length for predicting clean-fleece weight was slightly less than that obtained by scouring a small sample.

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A nomograph is presented for the rapid estimation of clean-fleece weight. Formulas are given for making annual corrections in the *a* values. These formulas account for annual variations in grease-fleece weight, staple length, and clean-wool yield as determined by scouring samples from a small group of ewes from each breed.

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RED STELE ROOT DISEASE OF THE STRAWBERRY CAUSED BY PHYTOPHTHORA FRAGARIAE[1]

By Henry F. Bain and J. B. Demaree, *senior pathologists, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

The root disease of strawberries that has been described as Lanarkshire disease (*24*),[2] red core root (*1*), black stele (*4*), red stele, [3] and brown stele (*21*), first attracted attention in Lanarkshire, Scotland, about 1920 (*3*, *24*, *25*). The first published report of the occurrence of the disease in the United States was from Illinois in 1935 (*4*). Recent reports indicate that the disease has become widely disseminated in both the British Isles and the United States. According to the latest available references, its present distribution is as follows: In Scotland, 18 counties (*18*), including practically all strawberry-growing districts (*2*); in England, 5 counties (*18*), including Kent, Hampshire, Devon, and Cornwall (*11*); in the United States, Illinois (*4*), Maryland,[3] New Jersey,[3] New York,[3] Michigan,[3] Virginia,[3,4] Delaware,[5] Indiana,[6] Kentucky,[7] Connecticut,[8] Wisconsin,[9] California (*21*), Oregon,[10] and Massachusetts [11]; in addition, the authors have received specimens of the disease from Arkansas, Ohio, Pennsylvania, Tennessee, and Washington.

Investigations into the nature of the Lanarkshire disease were initiated soon after its discovery in Scotland. Wardlaw (*24*, *25*, *26*) attributed the disorder primarily to poor cultural conditions that paved the way for the entry of weak root parasites, and O'Brien and M'Naughton (*17*) to parasitism by endotrophic mycorrhiza. Alcock and her coworkers (*1*, *2*, *3*) first noted the association with diseased roots of oospores and sporangia that resembled those of *Phytophthora*, correctly deduced that this *Phytophthora* was the true cause of the disease, gave a brief description of it on its host plant, but did not name it; their attempts to isolate and grow the organism in pure culture were unsuccessful. Anderson (*4*) first reported the black stele

[1] Received for publication May 11, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 505.

[3] Demaree, J. B., and Darrow, G. M. The red-stele root disease of strawberries in the northeastern United States. U. S. Bur. Plant Indus., Plant Dis. Rptr. 21: 394-399. 1937. [Processed.]

[4] Cook, H. T. Spread of strawberry red stele in Virginia. U. S. Bur. Plant Indus., Plant Dis. Rptr. 25: 296. 1941. [Processed.]

[5] Kadow, K. J. Strawberry diseases in Delaware. U. S. Bur. Plant Indus., Plant Dis. Rptr. 22: 184-186. 1938. [Processed.]

[6] Baines, R. C. Fruit diseases reported from Indiana. U. S. Bur. Plant Indus., Plant Dis. Rptr. 22: 335-336. 1938. [Processed.]

[7] Valleau, W. D. Red stele of strawberry in Kentucky. U. S. Bur. Plant Indus., Plant Dis. Rptr. 23: 153. 1939. [Processed.]

[8] Anonymous. Strawberry red stele appears in Connecticut. U. S. Bur. Plant Indus., Plant Dis. Rptr. 23: 249. 1939. [Processed.]

[9] Anonymous. Red stele of strawberry reported from Wisconsin. U. S. Bur. Plant Indus., Plant Dis. Rptr. 23: 259. 1939. [Processed.]

[10] Zeller, S. M. Strawberry diseases in Oregon in 1941. U. S. Bur. Plant Indus., Plant Dis. Rptr. 25: 512-513. 1941. [Processed.]

[11] Boyd, O. C. More about red stele disease of strawberries. Mass. Agr. Col., Ext. Crop Dis. Notes 6: 5-6. 1939. [Processed.]

root disease of strawberries in the United States from Illinois and emphasized its similarity to the Lanarkshire disease. Bain and Demaree (*6*) isolated the fungus causing red stele root disease of strawberries in Maryland, emphasized its marked resemblance to the *Phytophthora* associated with red core root in Scotland (*1*), and regarded the fungus as probably representing a new species of *Phytophthora*. Thomas [12] isolated an apparently identical *Phytophthora* in California from the disease later (*21*) described as brown stele of strawberries. In 1938 Hickman (*10*) isolated the causal fungus of red core root from strawberries growing in Kent County, England, and in 1940 (*11*) he described it as a new species, *P. fragariae*. Hickman's article appeared as the present authors were concluding an extended study of the American red stele pathogen, here reported upon and shown to be identical with *P. fragariae* Hickman.

THE DISEASE

Above-ground symptoms of the red stele disease are most evident in the spring and early summer and usually are first noted as severe dwarfing and death of plants in the lower, poorly drained parts of the fields. Individual plants appear to decrease in size as the older outer leaves wilt, dry up, and are replaced by smaller new leaves borne on shortened petioles. A characteristic bluish-green color of leaves is often associated with dwarfed plants. On warm, dry days in early spring there may be a sudden wilting of entire plants or of the outer leaves only, followed by partial recovery at night or on cooler days. Severely affected plants either bear no fruit or they bear very little and that of decidedly inferior quality. Often entire fruit clusters dry up after setting normal complements of berries but before bringing any of them to maturity.

These symptoms in the aerial parts of the plant result from physiological drought and starvation, brought about by progressive destruction of the major part of the root system by the pathogen. Young lateral roots are often completely rotted by the *Phytophthora* or by secondary fungi and bacteria, so that when diseased plants are dug the long primary roots may come out of the soil conspicuously free from fibrous roots; these constitute a highly characteristic feature of the disease aptly described as "rat's-tail" roots (*2*). The fungus has not been observed in roots more than 1 year old or in the crown.

As the fungus advances upward into the older parts of fleshy roots, the central cylinder is killed first, and the *Phytophthora* mycelium may be wholly confined to these tissues. Coincident with penetration, the stele turns a brownish-red color, producing an effect that has given rise to most of the common names for the disease. This reddened stele, very conspicuous against the background of whitish nondiseased cortical tissue in roots stripped or cut open longitudinally, may extend several inches above the dead lower end of the root (fig. 1). Exterior tissues of the root die belatedly after the invasion of the stele, either as the result of delayed penetration by *Phytophthora* or some weaker parasite or merely as the result of cessation of the nutrient supply. The dead lower ends of infected roots often turn an inky-black color.

The annual course of the red stele disease begins with the late-fall dying of the tips of roots and ends with the coming of hot weather in

[12] Personal communication.

late spring or early summer. During this period the roots which were first infected are gradually killed back to their origins at the crowns of the plants, while later infected roots may or may not be killed

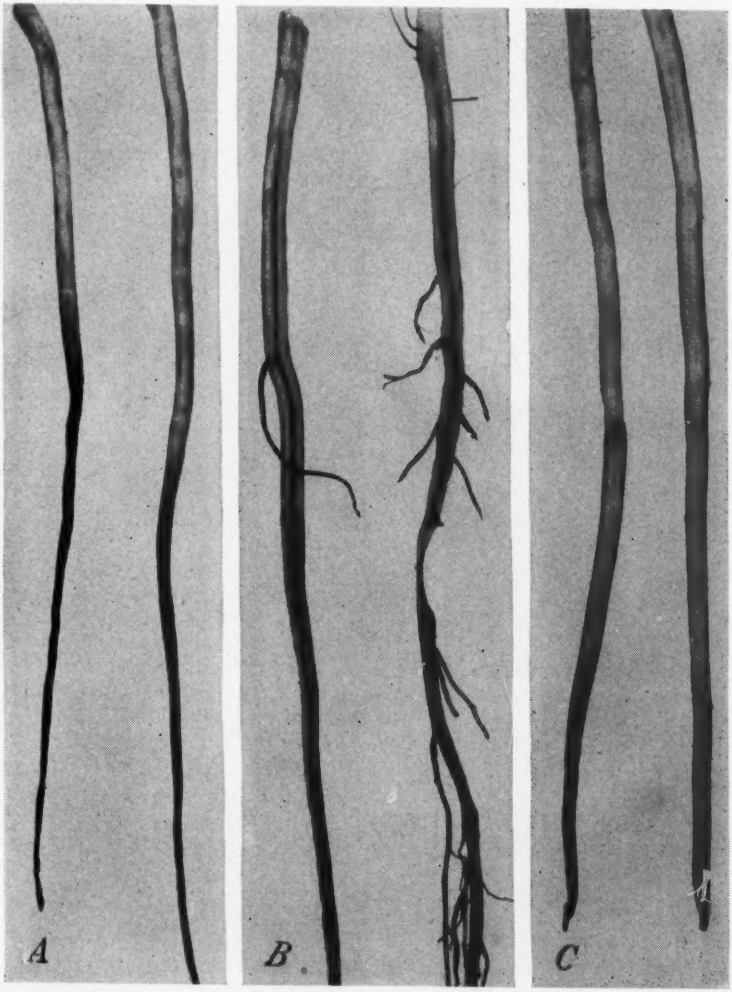

FIGURE 1.—Strawberry roots affected with the red stele disease. *A*, Diseased roots showing dead, discolored tip ends; upper parts of roots not discolored. *B*, Diseased roots split lengthwise to show reddened central portion. *C*, Normal roots split lengthwise to show absence of red center.

throughout their lengths before high temperature brings the disease to a complete stop. If a sufficient number of roots escape infection through the cool weather of spring, the plant may recover to some extent during the summer and develop new roots that remain free

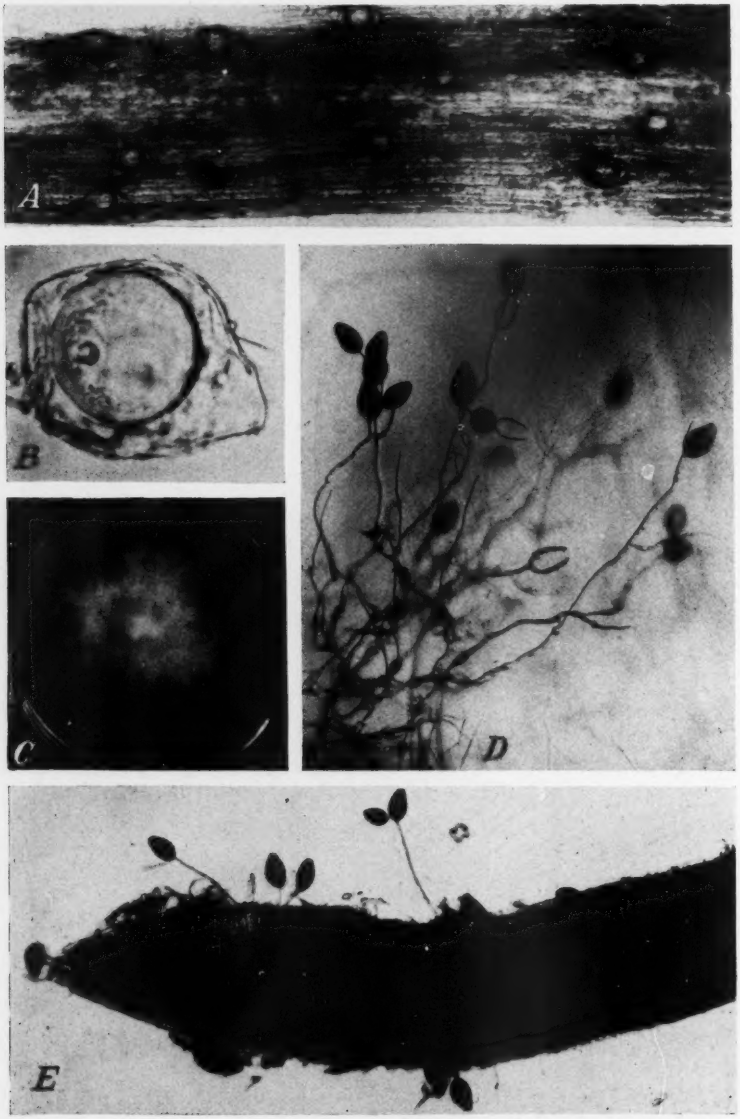

FIGURE 2.—*Phytophthora fragariae*. *A*, Oospores in dissected stelar root tissue. Oogonium walls enclosing the oospores are plainly visible in some cases. × 105. *B*, Mature oospore in oogonial envelope. Note the thick wall and the two spherical refractive bodies (one not in clear focus) at left edge of spore. × 750. *C*, 24-day-old culture on lima-bean agar. Approximately × 1/2. *D*, Mature and discharged sporangia from irrigated lima-bean-agar culture. Note proliferous type of branching in sporangiophore at top center. × 20. *E*, Sporangia on a diseased strawberry root tip. Upper right sporangiophore is sympodially branched. × 20.

from the disease as long as the soil temperature continues too high for the fungus to grow. This partial recovery is unavailing, however, as the fungus again becomes active with the advent of colder weather in the fall and the plant is attacked anew and more severely than before.

Positive diagnosis of the disease becomes much more difficult after the discolored-stele symptom is obliterated in early summer and can thereafter be made with certainty only by means of microscopic examination of roots. Thus, the period during which diagnostic macroscopic symptoms are present is relatively brief, a fact that should be particularly noted by nursery inspectors. Fall-dug plants may have incipient infections that easily escape detection because of their limited distribution and development, while it is almost impossible to diagnose light infections of the red stele disease during the summer months.

The most dependable proof that the red stele disease is present in suspected plants is the microscopic demonstration of oospores of *Phytophthora fragariae* in diseased roots. Oospores are produced almost exclusively in stelar tissue and as a rule are most abundant in the parts of the roots that were young when invaded. By making a thorough examination, the authors have never failed to find oospores in some part of every root known to be infected by the red stele organism. The oospores may be readily identified by their relatively large size, thick walls, and above all by the ever-present loosely fitting irregular-shaped oogonial envelopes, which enclose the oospores and impart to them a golden-brown or brick-red color (fig. 2, *A* and *B*).

THE PATHOGEN

The method of isolation of the cultures used in the present investigations has been described elsewhere (*6*) and need not be repeated here. A comparison of the authors' isolates with *Phytophthora fragariae* proved beyond question that the two are identical. After a comparative study of the group of *Phytophthora* spp. to which *P. fragariae* is most nearly related, and in view of the present chaotic state of classification within the genus, the authors agree with Hickman (*11*) that the strawberry red stele fungus should receive specific ranking, at least for the time being.

CULTURAL CHARACTERS

In its ability to grow in artificial culture media, *Phytophthora fragariae* appears to be intermediate between the majority of *Phytophthora* spp., which grow readily on a wide variety of artificial media, and the smaller group containing *P. phaseoli* Thax., *P. thalictri* G. W. Wils. and J. J. Davis, and *P. infestans* (Mont.) DBy., which grow much more slowly and on a more limited variety of media. Oatmeal agar, steamed bean pods, pea decoction, and lima-bean agar have proved favorable media, while the fungus either grows poorly or cannot be maintained at all after successive transfers on most common laboratory media such as corn-meal, potato-dextrose, and various malt-extract agars. Hickman listed Quaker-oat and French-bean agars as the most favorable media and lima-bean agar as fair and stressed as a classification feature the fact that the fungus does not grow on malt-extract agar.

The rate of growth of *Phytophthora fragariae* (fig. 3) is significantly slower than that of the following closely related species: *P. cryptogea* Pethyb. and Laff. (P–404, from Tucker through G. F. Gravatt), *P. cambivora* (Petri) Buisman (C–7, from Gravatt), *P. cinnamomi*

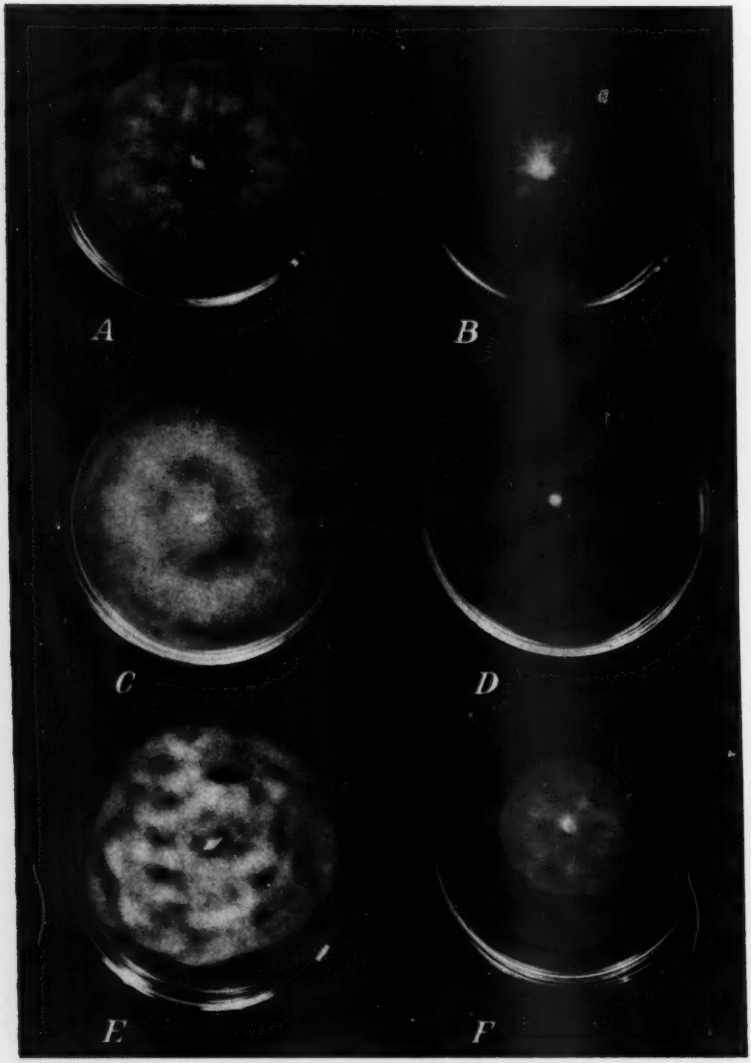

FIGURE 3.—*Phytophthora* cultures grown for 5 days at room temperature on lima-bean agar: *A*, *P. cryptogea* (P–404); *B*, *P. cambivora* (C–7); *C*, *P. cinnamomi* (B–5); *D*, *P. fragariae* (C–3); *E*, *P. drechsleri* (P–113); *F*, *P. megasperma* (P–340).

Rands (B–5, from Gravatt), *P. fragariae* (C–3, isolated from strawberry root at Beltsville, Md.), *P. drechsleri* Tucker (P–113, from C. M. Tucker), and *P. megasperma* Drechs. (P–340, from Tucker). Hickman (*11*) gave the rate of growth of *P. fragariae* as 20 mm. in 5 days (agar and temperature not specified). The authors' measurements to determine the effects of temperature on rate of growth, given in table 1, were made with lima-bean agar as the substrate; the fungus, however, grows at a more rapid rate on oatmeal agar, as may be seen by comparing tables 1 and 2.

TABLE 1.—*Relation of growth of Phytophthora fragariae (culture C–3) on lima-bean agar to temperature*

Temperature (°C.)	Diameter [1] of colony	
	After 7 days	After 14 days
	Mm.	*Mm.*
4	0	Trace
10	13	22
14	18	33
18	23	36
22	25	38
25	16	16
30	0	0

[1] Average of measurements of 3 colonies at each temperature, expressed as averages between longest and shortest diameters when colonies were irregular in shape, the usual condition.

TABLE 2.—*Relation of growth of Phytophthora fragariae (culture C–3) at 22° C. to initial pH of oatmeal agar*

Initial pH	Diameter [1] of colony	
	After 7 days	After 14 days
	Mm.	*Mm.*
4.0	3	50
4.6	30	70
6.0	34	78
6.2	39	81
6.8	40	82
7.0	43	85
7.2	38	76
7.6	37	65
7.8	0	Trace
8.0	0	0

[1] Each value represents the average of measurements of 3 colonies, as in table 1.

While it is, of course, unwise to attribute too much importance to the rate of growth on artificial media, nevertheless when collections of different species of *Phytophthora* are grown side by side one cannot fail to be impressed by the conspicuously slow growth rate of *P. fragariae*, and it is noteworthy that this slow rate is consistent in all isolations of the fungus so far made.

Table 1 also brings out a relation that is considered to have even greater definitive classification value by most students of *Phytophthora*, namely, the minimum, optimum, and maximum temperature points for growth of the fungus. In a collection of more than 30 species of *Phytophthora*, Leonian (*15*) found only 6 that failed to make some growth at 31° C.: *P. cactorum* (Leb. and Cohn) Schroet. (1 of numer-

ous strains), *P. citricola* Saw., *P. hibernalis* Carne, *P. porri* Foister, *P. richardiae* Buisman, and *P. syringae* Kleb. Tucker (*22, table 24*) found that of a large collection of species and strains of *Phytophthora*, only *P. erythroseptica* Pethyb., *P. hibernalis* (which he subsequently merged with *P. syringae*), *P. infestans*, *P. richardiae*, and *P. syringae* failed to grow at 30°. Only the last 4 of these species had optimum growth temperatures below 25°. The optimum for the authors' strain of *P. fragariae* was evidently between 18° and 22°, as the rate of growth had declined sharply at 25°. Hickman (*11*) gave the following temperature relations for *P. fragariae*: Optimum growth at 20°, death at 30°, fair growth at 10°, and scarcely any at 4°. Thus, the relatively low values for both optimum and lethal maximum temperatures serve to distinguish *P. fragariae* from all but a few other species of *Phytophthora*.

The effect of hydrogen-ion concentration on growth of the fungus was determined by using as the substrate oatmeal agar adjusted to different initial pH values with hydrochloric acid and sodium hydroxide. Petri-dish cultures of the fungus were grown for 2 weeks at a constant temperature of 22° C. Table 2 gives the results. Under the conditions prevailing, *Phytophthora fragariae* grew at pH values ranging from the minimum one tested, pH 4.0, to 7.6; it grew most vigorously and rapidly between 6.0 and 7.2.

MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS

Sporangia

Alcock (*1*) described the sporangia produced from diseased red core roots submerged in sterile water as follows: Nonpapillate, proliferous, usually inversely pyriform but variable in shape, variable in size, and averaging about 50μ by 30μ. Bain and Demaree (*6*) noted similar sporangia on diseased red stele roots and in irrigated pure culture of the fungus isolated from diseased roots.

Hickman (*11*) described the sporangia of *Phytophthora fragariae* as follows: Terminal on sporophores 10μ to 800μ long, nonpapillate, inversely pear-shaped, ovoid or ellipsoid, 32μ to 90μ by 22μ to 52μ, average 60μ by 38μ; both sympodial and proliferous types of branching of conidiophores present; sporangia produced in water, Petri solution, etc., but rarely on solid media.

As is to be expected, sporangia having the potentiality of reaching the unusually large size characteristic of *Phytophthora fragariae* vary considerably in both size and shape. In the authors' material, the sporangia emerging from diseased roots in tap water (fig. 2, *E*) measured 31μ to 70μ by 23μ to 38μ, and averaged 55μ by 33μ. In tap-water preparations of sporangia from mycelium grown on lima-bean agar (fig. 2, *D*), the corresponding dimensions were 39μ to 84μ by 28μ to 49μ and 65μ by 38μ, respectively. Figures 2, *D* and *E*, and 4, *A*, show some of the variations in size and shape. Malformed and dwarfed sporangia could be induced almost at will by growing the fungus in water from certain sources or by manipulating the temperature. Sporangia were never entirely normal in distilled water and often not in tap water, in which various amounts of chlorine and other solutes probably affected sporangial production. Water from melted snow proved excellent for inducing production of sporangia and zoospores. By using suitable water, the authors obtained zoospores in quantities

sufficient to inoculate several hundred potted strawberry plants without having to resort to the use of Petri solution or other similar methods for stimulating sporangial formation.

The effect of temperature on sporangial production was determined in routine preparations held continuously at various temperatures. The method of preparation suggested by Charles Drechsler, of this Division, was as follows: After the fungus had grown for 2 weeks or longer on thin layers of lima-bean agar in Petri dishes, the growth masses were cut into 5- by 10-mm. rectangular pieces, 20 or 25 pieces were transferred to a Petri dish, and sufficient water was added to barely cover the thinnest agar blocks in the dish. Sporangia developed most abundantly on sporangiophores growing into the water from the

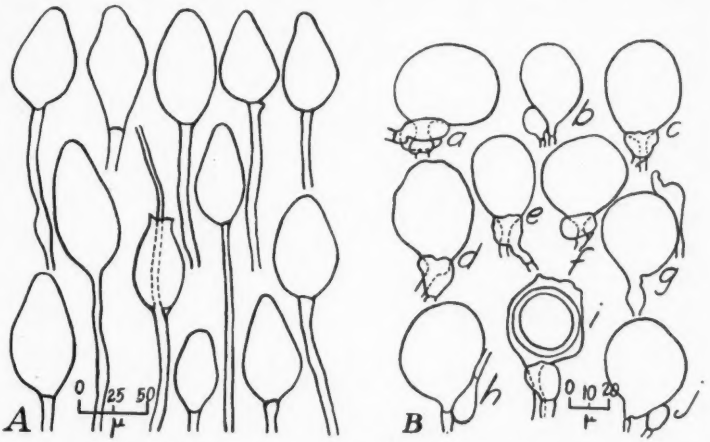

FIGURE 4.—*Phytophthora fragariae: A*, Sporangia, showing range in size and shape, × 200; *B*, *a–j*, oogonia and antheridia, × 300.

edges of the blocks (fig. 2, *D*), although sometimes they were produced copiously over the entire upper surface. Sporangial production was most vigorous and abundant at 14° C., slightly less so at 18°, fairly abundant at 10° and 22°, and occasional at 25°. At the last-mentioned temperature there was no zoospore discharge, and all sporangia eventually disintegrated completely. Not many sporangia discharged zoospores at 22°. The optimum temperature for sporangial and zoosporic production thus appears to be several degrees lower than the optimum for mycelial growth. At 22° and 25° sporangia began to appear 1 or 2 days after the preparations were set up, while at the lower temperatures 3 or 4 days, sometimes even longer periods, were required. If the water was changed daily at the favorable temperatures, sporangia continued to form for 2 weeks or longer. Some evidence was obtained that fluctuating temperatures may be more conducive to sporangial and zoosporic production than constant temperatures.

It remains controversial whether sporangial characters in *Phytophthora* spp. have much classification value, but most investigators

agree that the presence or absence of a papilla separates the species into two general groups. On this basis, *P. fragariae* would be grouped with the nonpapillate species *P. cambivora*, *P. cinnamomi* (Mehrlich (*16*) advocated merging *P. cinnamomi* with *P. cambivora*), *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, *P. hibernalis* (synonymous with *P. syringae* according to Tucker (*22*)), *P. megasperma*, *P. porri*, *P. richardiae*, and *P. syringae*.

Zoospores

As noted by Hickman (*11*), *Phytophthora fragariae* has zoospores typical of the genus; they are irregularly ellipsoidal, grooved on one side, and biciliate, round up on resting, are 12μ in diameter, occur generally 40 to 50 per sporangium, and germinate by germ tube, sometimes in the sporangium. To this description the authors can add little. Zoospores were usually about 10μ in diameter after coming to rest.

To determine the duration of the free-swimming period of zoospores, the authors transferred a concentrated suspension that had been discharging from sporangia since 11 a. m. to a Petri dish at noon and held it at 10° to 15° C. for the duration of the test. At 1:30 p. m. the spores were still swimming freely. At 2 p. m. most were swimming, some had encysted, and a few had already germinated. At 3:30 p. m. there was a conspicuous reduction in numbers and activity of swimming zoospores, but the percentage of germination in encysted spores was not high. The last observation was made at 4:15 p. m., when only an occasional spore could be found swimming. Germination was still poor.

Although the authors did not succeed in determining temperature limits for zoospore germination, they found germinated spores most often at 10° and 14° C. Zoospores are evidently exceedingly sensitive to the temperature as well as to the composition of the liquid in which they are released.

A final observation appears worth recording. On one occasion when a large number of zoospores was desired, a heavy crop of sporangia developed in the daytime and appeared likely to mature and discharge spores during the night. In an attempt to arrest the discharge, the whole lot, consisting of some 50 Petri-dish preparations, was poured into a 2-liter beaker, which was then filled with water and placed outside the laboratory window at near-freezing temperature. No swarm spores had been discharged at the end of the working day. When the beaker was examined the following morning, a thin film of ice covered the surface of the water, but most of the sporangia had nevertheless succeeded in discharging zoospores, vast numbers of which were actively swimming in the icy water.

The Sexual Stage

It is almost impossible to obtain satisfactory material for precise morphological study of the sexual stage of *Phytophthora fragariae*. Hickman (*11*) stated that in his material the fungus occasionally produced a few scattered oospores in oatmeal-agar cultures, but that such spores were too closely associated with fragments of oat kernels or hulls to be of much value for morphological study; he made no mention of oospores in or on any other medium. In the authors' isolates the

sexual stage failed to develop in any of the artificial media used, except in old oatmeal-agar cultures and in steamed string beans, where a few red oogoniumlike bodies of indeterminate structure could be found. Mating different isolates of the fungus yielded no evidence of heterothallism; on the contrary, the unmated strains invariably produced oospores when inoculated separately into their natural medium, the roots of growing strawberry plants. Inability of *Phytophthora* cultures to produce the sexual stage in artificial media is of course considered to be a classification character of some importance, but unfortunately it limits the study of this stage of the fungus to material occurring naturally in the host plant.

Sexual organs of the red stele root disease fungus as a rule develop only in central cylinders of infected roots. This region of compact cellular tissue is stained so deeply as a result of fungus penetration that minute details of young reproductive structures cannot be made out in fresh material. Prepared slides proved equally unsatisfactory for studying the process of sexual reproduction.

The best results were obtained in whole mounts of steles dissected free from the cortex and decolorized with a weak bleaching-powder solution, then stained very lightly with gentian violet, cotton blue, or some similar stain, and cleared in dioxane and mounted in water or cleared and mounted in lactophenol solution. Both treatments disorganized the oily contents of the reproductive organs but left the cell walls intact in their natural positions. The relation between antheridia and oogonia could be most reliably made out in the early stages of fertilization, before oospores were delimited within the oogonia. In figure 4, *B*, only 1 oogonium (*i*) out of the 10 illustrated contains a mature oospore.

Study of material prepared as described raised a question whether the antheridia are predominantly amphigynous as first stated by Alcock (*1*) and later by Bain and Demaree (*6*) and Hickman (*11*). It is true that antheridia are usually closely attached to the bases of oogonia, but after prolonged search the authors did not find a single instance in which they could be sure that the oogonial stalk actually penetrated the antheridium. On the contrary, it is not difficult to find favorably oriented material in which the association is unmistakably paragynous (fig. 4, *B*, *b*, *g*, *h*, *j*). Two cases that might possibly have been amphigynous are illustrated in figure 4, *B*, *a* and *f*. In the other illustrations (fig. 4, *B*, *c*, *d*, *e*, *i*), which are typical of most cases seen in roots, the antheridia were situated entirely above the oogonial stalks, although at first sight they appeared to be amphigynous. Alcock, Howells, and Foister (*3*) eventually concluded that the two types of fertilization take place in about equal proportions. Satisfactory study of minute details of fertilization must await the discovery of a medium other than living roots in which sexual reproduction will take place freely.

Grosser reproductive structures are easily made out. Hickman's (*11*) description may be summed up as follows: Oogonia terminal or lateral, variable in shape, 28μ to 46μ (average 39μ) in diameter, wall thick, smooth, golden brown with age; antheridia usually terminal, amphigynous or less commonly paragynous, 16μ to 30μ by 12μ to 22μ (average 22μ by 16μ); oospores spherical, ellipsoidal or more irregular, diameter of spherical spores 24μ to 44μ (average 33μ); oospore wall 3μ

thick; oospore containing a single large central oil globule. The authors consider that this central area is a vacuole, not an oil globule.

The oogonia tend toward ellipsoidal to subglobose shapes in the earlier stages, but they accommodate themselves somewhat to the limitations of the crowded region in which they develop (figs. 2, *A* and *B*, and 4, *B*). After the oospores are differentiated, the oogonia lose their turgidity, but the partially collapsed walls persist as tough, loosely fitting envelopes, which in time become dark red to golden brown. In the authors' material 125 oogonia measured 29μ to 75μ by 26μ to 52μ and averaged 55μ by 39μ.

The spore walls are perfectly smooth and the spores themselves are colorless, as may be demonstrated by dissecting off the colored oogonial envelope; in mature spores there are typically 2 lenticular "refractive bodies," 3.5μ to 6.5μ in maximum diameter, embedded in the peripheral cytoplasmic layer (fig. 2, *B*). In the authors' material 200 mature spherical oospores ranged from 20μ to 46μ in diameter and had a mean of 33.5μ. Elliptical spores usually occurred in the ratio of 1 to 4, and the maximum deviation from spherical shape was a 20-percent difference in lengths of major and minor axes.

The sexual characters most commonly considered to have taxonomic importance in *Phytophthora* spp. are type of antheridium, size of oospore, and ability of the fungus to produce the sexual stage in artificial media, either directly or after certain manipulations. As knowledge concerning the behavior of *Phytophthora* spp. gradually accumulates, these characters are frequently found to vary more within described species than between species, resulting in much taxonomic confusion, and indeed leading Leonian (*15, p. 33*) to hold that there are probably not more than three true species in the genus.

In the characters just mentioned, the red stele fungus compares with its most nearly related species as follows: If antheridia should prove to be always predominantly paragynous, the fungus would belong with the group containing *Phytophthora syringae*, *P. hibernalis*, *P. porri*, and *P. megasperma*. These four species, like *P. fragariae*, have unusually low temperature-growth relations. (Tucker (*22*) reduced *P. hibernalis* to synonymy with *P. syringae;* the description of *P. porri* (*9*) was evidently not available at the time of his publication. Leonian (*15*) retained the first three species, but recognized their close relationship. The temperature relations of *P. megasperma* are given by Wager (*23, table 3*).) If, on the other hand, the red stele fungus eventually proves to be predominantly amphigynous, as it has usually been considered up to the present time (*1, 6, 11*), it would be grouped with *P. cambivora*, *P. cinnamomi*, *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, and *P. richardiae*.

Previous to the description of *P. fragariae*, only two species of *Phytophthora* having oospores with an average diameter greater than 30μ in the respective host plants had been described. They were *P. megasperma* (*8*) in the paragynous group, with oospores averaging 41μ in diameter, and *P. erythroseptica* in the amphigynous group, whose oospores have been variously described with 31μ to 37μ average diameters. In these two species, spore size has been considered a valid diagnostic character. If this precedent is followed, the oospore size of *P. fragariae* (about 33μ average diameter) must be considered sufficiently distinctive to separate it from all described species of

Phytophthora except *P. erythroseptica*. Indeed, Tucker (*22, p. 180*) suggested that these two species might be identical merely on the basis of Alcock's original description of the red core root fungus from its host plant (*1*). However, even if *P. fragariae* eventually proves to belong in the amphigynous group with *P. erythroseptica*, certain cultural and host relations recounted later appear to warrant separating the two species.

According to Tucker (*22*), *Phytophthora erythroseptica* produces oospores rather abundantly in old oatmeal-agar cultures; Rosenbaum (*19, table 1*) stated that oospores were produced on a number of media; Leonian (*15, table 4*) found that oogonia developed when mycelial mats grown in pea broth were transferred to distilled water. In its ability thus to produce oospores, *P. erythroseptica* differs from *P. fragariae*, for, as has been pointed out previously, the latter consistently fails to develop the sexual stage on artificial media under these as well as all other conditions tried, except for the sparse production of doubtfully functional oosporelike structures in old oatmeal-agar cultures and on steamed string beans. The temperature-growth relations of the two species are also significantly different.

The authors observed oospore germination in only 1 instance. In February 1940, 50 oogonia containing mature oospores were dissected from diseased roots and after being transferred to hanging-drop water cultures in 5 Van Tieghem cells were kept in a refrigerator at a temperature of 10° C. No germination was noted during the next 3 months. The slides were then left undisturbed in the refrigerator until early August. At that time only 1 slide had not dried out. In it 1 spore had germinated sometime previously, giving rise to a long-stalked sporangium which had discharged its contents and had been followed by 1 additional proliferous sporangium which likewise appeared to have discharged zoospores in a normal manner. Several recently formed sporangia were found at the same time in some water-agar Petri-dish preparations of similar age on which entire steles instead of isolated oospores had been placed. These sporangia probably came from germinated oospores, but connections between sporangia and oospores could not be traced with certainty.

The authors have made continuous but wholly unsuccessful efforts to follow the entire course of development of oospores in strawberry roots. The spores obviously require a resting period before germinating, as roots infected during the fall-winter-spring period contain a high proportion of normal-appearing oospores throughout the succeeding summer. Under field and greenhouse conditions, such roots have largely rotted away by the following spring and it is rare to find normal-appearing spores in the few that remain. During the fall season, after the summer rest period, the proportion of apparently normal spores in these old roots decreases rapidly, presumably in large measure as the result of germination.

Oospore behavior is unquestionably of extreme importance to the incidence and perpetuation of the disease. Throughout the greater part of the United States summer temperatures are undoubtedly high enough to kill out the mycelial stage of the fungus, and the oospores consequently must serve to oversummer rather than to overwinter the disease. The maximum temperature that oospores are able to withstand is not known and cannot be determined until a

reliable method of germinating the spores is discovered; indirect evidence on this point, however, is presented below under the account of a hot-water-treatment experiment. It is also noteworthy that the disease invariably recurs in potted plants held over summer in the greenhouse at the Plant Industry Station, Beltsville, Md., where for weeks at a time the daytime temperatures to which the pots are subjected vary from 90° to well over 100° F. Judging from the comparatively narrow range of artificial media on which the fungus thrives, from its inability to produce oospores in the absence of its host plant, from the sensitivity of its mycelium to high temperatures, and from its limited host range (p. 502), it appears unlikely that the fungus can maintain itself in the soil if strawberry plants are not available at the critical period when oospores are germinating. Thus longevity of oospores may well determine the length of time the disease can persist in soils kept free from strawberry plants.

INOCULATION EXPERIMENTS

PATHOGENICITY OF PHYTOPHTHORA FRAGARIAE TO CULTIVATED STRAWBERRY

Since the authors have already presented evidence establishing the pathogenicity of their strains of *Phytophthora fragariae* (*6*), it is unnecessary to describe such experiments in detail here. Figures 5 and 6 illustrate the results obtained in an early experiment of this sort,

FIGURE 5.—*A*, Strawberry plant 3 weeks after inoculation with *Phytophthora fragariae*. *B*, Noninoculated check plant.

in which zoospore suspensions in sterile water were poured over the roots of young strawberry plants of the variety Blakemore growing in pots in autoclaved soil. After inoculation these plants were held for 3 weeks in fairly wet soil in a greenhouse at a temperature of 50° to 60° F. As a consequence of the mass inoculation, all main roots on these small plants became infected simultaneously and the wilting

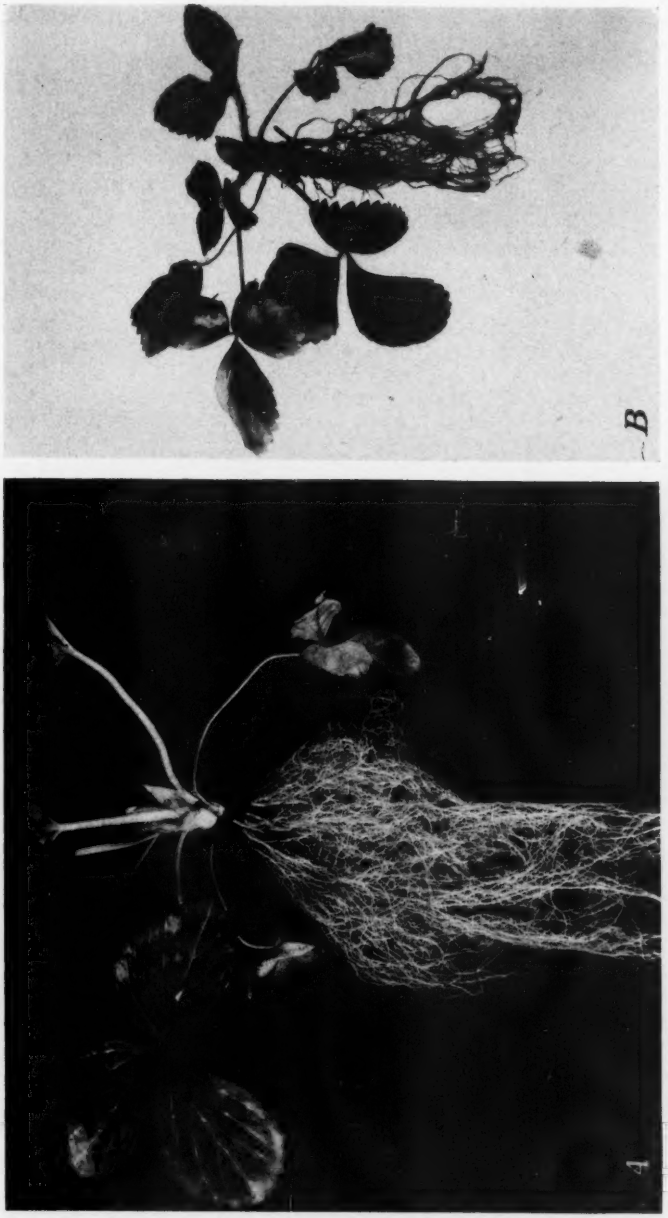

FIGURE 6.—Plants from the same lot as those shown in figure 5: *A*, Noninoculated plant; *B*, inoculated plant, showing destruction of root system by the pathogen.

stage was reached in the short time of 3 weeks. *P. fragariae* was reisolated from the roots of the inoculated plants.

The isolate of *Phytophthora fragariae* used most frequently (C–3, isolated in November 1937) has been passed through and reisolated from strawberry roots 4 years in succession. When subcultures freshly recovered from strawberry roots were compared with older isolates, it was found that in culture the fungus gradually lost some of its capacity for producing sporangia but that the zoospores produced retained their pathogenicity unimpaired.

HOST RANGE OF PHYTOPHTHORA FRAGARIAE

Species Other Than Fragaria

Certain selected plants unrelated to *Fragaria* were inoculated to obtain information on the host range of *Phytophthora fragariae* and to compare it taxonomically with other *Phytophthora* spp. The method of inoculating roots of potted plants was to place blocks of mycelial tissue from Petri-dish cultures directly on the roots after the plant was removed from its pot, or, much more frequently, to pour a suspension of swimming zoospores over the roots; the plant was then returned to the pot and kept fairly wet in a cool greenhouse (55° to 65° F.). When parts other than roots were being inoculated, mycelium in masses sufficiently large to assure an abundance of viable inoculum was placed in incisions that were then either sealed with paraffin or wrapped with moist sterilized cotton and enclosed in Parafilm. Each test was usually made in triplicate and in some cases the same experiment was repeated several times. Because of the slow growth rate of the fungus, at least 2 weeks was allowed to elapse before final records of the results were taken.

Phytophthora fragariae failed to infect the following:

Potato tubers.—*P. erythroseptica* and *P. drechsleri* were originally described from this host; and, according to Tucker (*22*), *P. cinnamomi* and *P. cryptogea* are also pathogenic to the tubers.

Calla lily (roots of potted plants).—*P. richardiae* was described from this host and is said to be virulently pathogenic to it.

Leek leaves and roots.—The native host of *P. porri* (*9*).

Tomato seedlings and green fruits.—*P. cryptogea* was originally described from tomato stems and roots. Tucker (*22*) listed *P. cryptogea* and *P. drechsleri* as virulently pathogenic to tomato seedlings and *P. cryptogea* and *P. erythroseptica*, to green fruits; he listed *P. drechsleri* and one strain of *P. cinnamomi* as weakly pathogenic to the fruits.

Apple fruits.—Mycelium of *P. fragariae* grew a short distance into the tissues of one ripe Grimes Golden apple without causing rot, but it failed to grow at all in several others. Tucker's group virulently pathogenic to apple contains *P. cinnamomi*, *P. hibenalis*, and *P. syringae* and the weakly pathogenic group *P. cambivora*, *P. cryptogea*, *P. erythroseptica*, and *P. drechsleri* (*22*).

Eggplant fruits.—Tucker's virulently pathogenic group contains *P. cryptogea* and *P. drechsleri* (*22*).

Beet roots, green lima-bean pods, bean stems and roots, roots of *Potentilla monspeliensis* L., pansy roots, carrot roots.

Thus, *Phytophthora fragariae* has not infected the living tissues of any plant species so far tested outside of the genus *Fragaria*. Among the plants proved nonsusceptible there is at least one which is susceptible to each previously described nonpapillate species of *Phytophthora* except *P. megasperma*. The authors have not inoculated hollyhock, the native host of *P. megasperma*, with *P. fragariae*.

Species and Varieties of Fragaria

The following species of *Fragaria* proved highly susceptible when inoculated with *Phytophthora fragariae*: *F. moschata* Duch., *F. virginiana* Duch., two strains of *F. vesca* L., and two of three strains of *F. chiloensis* (L.) Duch. The third strain of *F. chiloensis* did not become infected.

Some commercial strawberry varieties are known to be highly resistant to the red stele root disease (*14*, *18*, *20*). Of these, only the Aberdeen has so far been tested for resistance by direct zoospore inoculation of potted plants. Plants of this variety have never become infected despite repeated efforts to transmit the disease to them, although Anderson and Colby [13] have recently reported finding it slightly infected in the field in Illinois. Four resistant varieties developed in Scotland (*18*) and brought to this country for breeding purposes under P. I. [14] Nos. 127389 to 127392 have been made available through the courtesy of H. W. Anderson, of the University of Illinois. These, likewise, have proved wholly resistant when inoculated heavily with zoospores. The pot-inoculation technique is being used to supplement field tests in determining red stele resistance of hybrid seedlings now being developed in the strawberry breeding project conducted jointly by George M. Darrow, of this Division, and W. F. Jeffers, of the University of Maryland (*13*, *14*).

Pathogenicity of Related Species of Phytophthora to Strawberry Roots

Groups of three potted Blakemore plants were repeatedly inoculated with mycelium, and with zoospores when available, of the nonpapillate species *Phytophthora cinnamomi*, *P. cryptogea*, *P. drechsleri*, *P. erythroseptica*, *P. fragariae*, and *P. megasperma*. The three plants inoculated with *P. fragariae* became infected and developed characteristic red stele disease symptoms. The remaining fungi without exception failed to infect roots of the plants on which they were placed. Noninoculated check plants likwise remained disease-free.

Cultures of the remaining nonpapillate species of *Phytophthora* either were not available or for other reasons were not tested against strawberries.

Experimental and Practical Control

In common with many root troubles, the red stele root disease in strawberries cannot be economically controlled by any known direct treatment of infected soil or diseased plants, but the problem of control must be approached indirectly by such means as growing resistant varieties, rotation of crops, fallowing infected fields as long as may be necessary to rid them of the fungus, and regulating the distribution of planting stock by individuals and more particularly by nurseries. Development of resistant strains by breeding appears to offer the most promising prospect of eventual commercial control of the disease. Various groups of plant breeders both here and abroad are at present engaged in the endeavor (*5*, *7*, *12*, *13*, *14*, *18*).

It is not definitely known how long *Phytophthora fragariae* is able to persist in infected soil kept free from its only known host plant.

[13] Anderson, H. W. and Colby, A. S. Red stele of strawberry on Pathfinder and Aberdeen. U. S. Bur. Plant Indus. Plant Dis. Rptr. 26: 291–292. 1942.

[14] "P. I." refers to the accession numbers of the Division of Plant Exploration and Introduction.

Alcock and Howells (*2*) made some observations which indicated that the survival period might be 8 years or longer in Scotland. Some theoretical considerations discussed previously, based on temperature (p. 493) and nutritional reactions of the fungus, make it appear improbable that the fungus can survive for such long periods in most regions of the United States. A small test, started in May 1939, designed to furnish more definite data about the longevity of the fungus in infested soil, has already given some information. In that year strawberry plants well infected with *Phytophthora* incorporated with soil were placed in a shallow excavation in a field adequately isolated from growing strawberries. In March of the following year a sample of the soil was withdrawn, potted, and planted to 10 disease-free strawberry plants. In November of the same year, or 18 months after the test was started, another sample of soil was taken in which 20 plants were set. Both lots of plants were examined in April 1941. All 30 plants were badly infected with the red stele root fungus. In the autumn of 1941, or after a lapse of 2½ years, a third sample was taken in which 20 nondiseased plants were set. In this lot 1 plant only showed a trace of the disease the following spring (1942). None of the plants used as controls with the 3 lots developed the disease.

The authors are constantly on the lookout for some safe treatment of dug nursery plants that will kill the fungus in the root tissues of lightly infected plants which may have escaped detection during nursery inspection. Fungicidal treatments of this nature are carried out in cooperation with M. C. Goldsworthy, of this Division. So far no effective treatment has been found.

That hot-water treatments are likewise ineffective was proved experimentally by immersing badly diseased plants at different temperatures for different lengths of time and then potting an untreated disease-free plant with each treated plant in autoclaved soil. The experiment was started in the spring and the plants held under conditions favorable for both plant growth and fungus development until the following spring. Temperatures of 90°, 100°, 110°, 115°, and 118° F. were used, and time of immersion was varied from one-fourth hour to 5 hours. In all, there were 25 different temperature-duration series. Each series was replicated 10 times. Of the treated plants, 38 percent died, presumably as a result of the treatment. The roots of the dead plants were left undisturbed in the pots with the companion plants. The criterion of the effect of the treatments was the absence of the root fungus in the roots of the control plants at the termination of the test. The results of the test indicated that the pathogen was slightly more tolerant to the treatments than the plants. For evidence to support this conclusion, 2 examples may be cited. In plot 16, held at 110° for 2 hours, 8 of the 10 treated plants died, but the fungus spread to 5 of the control plants. In plot 18, held at 110° for 4 hours, all treated plants died, but 1 of the companion plants contracted the disease. At least 1 control plant of the 10 in each of the 25 temperature-duration series contracted the disease.

The red stele disease has so much potentiality for harm and is so difficult to cope with when once introduced that it is impossible to place too much emphasis on the importance of preventing its introduction into noninfested strawberry regions. With the present wide

distribution of the disease in the United States, this can be accomplished best by vigorous educational programs in conjunction with efficient inspection and certification systems applied to all nurseries and individuals distributing strawberry plants.

SUMMARY

The fungus causing the strawberry root disease known in the United States as black stele, red stele, or brown stele is shown to be identical with *Phytophthora fragariae* Hickman, which causes a similar strawberry root disease in Scotland and England known as Lanarkshire disease or red core root.

In this country the disease has been found in the following States: Arkansas, California, Connecticut, Delaware, Illinois, Indiana, Kentucky, Maryland, Massachusetts, Michigan, New Jersey, New York, Ohio, Oregon, Pennsylvania, Tennessee, Virginia, Washington, and Wisconsin.

Morphological and physiological studies of the fungus associated with the disease in the United States are described in detail.

Inoculation of roots, leaves, or fruits of plants other than strawberry known to be hosts to other *Phytophthora* spp. indicated *Fragaria* to be the only plant genus susceptible to infection by *P. fragariae*, and inoculations within the genus *Fragaria* showed that species and horticultural varieties of the cultivated strawberry vary in susceptibility to attack by the fungus.

The only practical methods of controlling the disease known at the present time consist in growing resistant varieties, keeping fields free of strawberries for at least 3 or 4 years after the soil has become contaminated, and preventing introduction of the disease into noncontaminated soils.

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